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Anti-Ro52 antibody is an independent risk factor for interstitial lung disease in dermatomyositis

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ABSTRACT

Aim: Recent studies have shown that *anti*-Ro52 antibody is associated with both interstitial lung disease (ILD) and the degree of disease severity in juvenile patients with dermatomyositis (DM). We found that more than half of adult patients with DM were positive for *anti*-Ro52 antibody. In this study, we analysed the correlation between *anti*-Ro52 antibody and ILD in adult patients with DM.

Method: Serum samples were collected from 153 adult inpatients with DM, at the First Medical Centre of PLA General Hospital, Beijing, China, who met the classification criteria of idiopathic inflammatory myopathies from March 1, 2016 to September 30, 2019. The patients were followed up to May 31, 2020. Immunoblotting was used to detect 16 types of myositis-specific autoantibodies (MSAs) and myositis-associated autoantibodies (MAAs) from serum samples. High-resolution computed tomography (HRCT) was used to calculate the ILD score, and tumours were screened. Clinical data and HRCT scores were evaluated and analysed retrospectively.

Results: Our results showed that *anti*-Ro52 antibodies were the most commonly found antibodies in patients with DM, with a positive rate of 52.9%. *Anti*-Ro52, *anti*-aminoacyl-tRNA synthetase (anti-ARS), and anti-melanoma differentiation-related gene 5 (*anti*-MDA5) antibodies were found to be risk factors for ILD development. *Anti*-Ro52 antibodies had a strong predictive effect on ILD in patients with DM.

Conclusion: The occurrence of ILD is highly likely in patients with DM who are positive for the *anti*-Ro52 antibodies. Thus, *anti*-Ro52 antibodies is an independent risk factor for ILD in patients with DM.

1. Introduction

Dermatomyositis (DM) is an autoimmune connective tissue disease involving the skeletal system, muscle, skin, lungs, and other organs. DM is associated with a high mortality rate, and the main factors affecting its prognosis include secondary infection, interstitial lung disease (ILD), and malignant tumours [1]. Previous studies have shown that the incidence of ILD in patients with DM is 19.9–78% [2]. Recent studies have shown that myositis-specific autoantibodies (MSAs) and myositis-associated autoantibodies (MAAs) have a certain predictive value for the prognosis of DM [3]. Anti-melanoma differentiation-related gene 5 (*Anti*-MDA5) antibodies are expressed in certain DM patients with rapidly progressive ILD and associated with a high mortality rate [4]. Antiaminoacyl-tRNA synthetase (anti-ARS) antibodies were detected in 15–30% of patients with DM with higher incidences of ILD [5]. Recent studies have shown that *anti*-Ro52 antibodies are associated with ILD and the degree of disease severity in juvenile patients with DM [6]. One study reported that 6 adult patients with DM with isolated *anti*-Ro52 antibodies developed rapidly progressive ILD, but they responded well to therapy and their prognosis were good [7]. However, we found that more than half of adult patients with DM were positive for *anti*-Ro52 antibodies. The significance of *anti*-Ro52 antibodies in adult patients with DM remains unclear. In this study, we analysed the correlation between *anti*-Ro52 antibodies and ILD in adult inpatients with DM.

2. Methods

2.1. Study cohort and patients

Our study retrospectively collected data from all patients with DM in the inpatient department at the first medical centre of PLA General

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Hospital, Beijing, China from March 1, 2016 to September 30, 2019. The patients were followed up to May 31, 2020. A total of 153 patients with DM were selected and analysed. Serum samples of patients were collected for autoantibody testing when they were diagnosed as DM, for the first visit to our hospital. This study was approved by the Research and Ethical Review Committees of PLA General Hospital. Informed consent was obtained from all the patients and/or immediate relatives, according to the Declaration of Helsinki.

The data collected included age at onset, sex, the course of disease, follow up time, main symptoms, important physical signs, and abnormal laboratory indicators including lactate dehydrogenase (LDH) and creatine kinase (CK). Complications included tumours and ILD. High-resolution computed tomography (HRCT) was used to evaluate ILD and the tumours were screened using serum tumour markers, systemic superficial lymph node ultrasound, abdominal enhancement magnetic resonance imaging (MRI), nasopharyngeal endoscopy, gynaecological ultrasound, gastrointestinal endoscopy, and/or systemic positron emission tomography–computed tomography (PET-CT).

2.2. Inclusion and exclusion criteria

All the patients met the classification criteria of idiopathic inflammatory myositis (IIMs) in 2017 EULAR/ACR [8]. The definite all of 153 DM patients >90%. The total score was ≥7.5 in patients without muscle biopsy and ≥8.7 in patients with muscle biopsy. Excluding polymyositis (PM), immune-mediated necrotising myopathy (IMNM), inclusion body myositis (IBM), juvenile myositis and juvenile dermatomyositis (JDM). And meanwhile excluding the overlap syndrome, metabolism-related myopathy, and drug-related myopathy.

2.3. Detection of serum autoantibodies

Sixteen types and levels of MSAs and MAAs were collected: MAAs including antibodies PM-Scl75, PM-Scl100, SRP, Ku, and Ro52; Anti-ARS antibodies, including antibodies Jo-1, PL-7, PL-12, EJ, and OJ; and other MSAs including antibodies MDA-5, TIF1-γ, NXP2, Mi-2α, Mi-2β, and SAE1.

Sixteen type of MSAs and MAAs were detected via immunoblotting. Serum (15 μL) was taken from each patient and diluted uniformly with 1.5 mL sample buffer. The numbered side of the test strip was placed upside down in the incubator, and 1.5 mL of sample buffer was added into each incubator, incubated on the shaking bed at room temperature (18–25 °C) for 5 min, then, the liquid in the incubator was absorbed and the diluted serum samples were added into the incubator. After incubating at room temperature for 30 min, the film strips were cleaned 3 times with 1.5 mL buffer solution, for 5 min each time. Then, the diluted enzyme conjugate (*anti*-IgG labelled with alkaline phosphatase) was added to the incubator. The buffer was again incubated for 30 min at room temperature before cleaning, as previously. Then, the substrate solution (1.5 mL) was added in the incubator. After incubation at room temperature for 10 min on a rocking bed, the film strips were cleaned 3 times with distilled water, for 1 min each time. Finally, the test strip was placed in the result determination template, and the result was determined by the EUROLineScan program (3.2.1), after air drying.

2.4. The HRCT scores of ILD in patients with DM

The classical six-point method was used to calculate the HRCT scores. When the patient with DM first came to our hospital to be hospitalized, as described below. The lung structure was divided into six regions: upper left, upper right, middle left, middle right, lower left, and lower right. The upper region was the tracheal carina, the lower region was the pulmonary vein, and the rest was the middle lung. Pulmonary structural changes and scores included: (1) attenuation of normal signals, with a score of 1; (2) ground glass changes (GGO) without bronchiectasis, with a score of 2; (3) consolidation without bronchiectasis, with a score of 3; (4) ground glass changes with bronchiectasis, with a score of 4; (5) consolidation with bronchiectasis, with a score of 5; (6) alteration of lung honeycomb, with a score of 6. The six regions were independent of each other, with 5% as the unit. The range of abnormal images involving the lungs was estimated separately, and then multiplied by the corresponding scores. Subsequently, the average of the six regions was taken as the final HRCT score of each patient [9]. Two specialists, who have been carrying out imaging work for 5 years, completed the evaluation of HRCT scores, and the scores from these 2 doctors were averaged.

2.5. Statistical analysis

For binary data group comparisons, χ^2 or Fisher's exact tests were used. Comparisons of continuous data were made using the Student's t-test. Risk factors were analysed via logistic regression. Kaplan-Meier survival curve was used to compare the survival and death cases. A *P*-value <0.05 was considered significant.

3. Results

3.1. Patient characteristics

In this study, ILD was detected in 80 of 153 patients (52.3%; ILD presence group) and undetected in 73 patients (47.7%; ILD absence group). While both groups had more female than male patients, their ratio was not significantly different ($P = 0.580$). Age at DM onset was also not significantly different between the groups ($P = 0.074$). The median age of all DM patients was 48 years, and there was no difference in the incidence of ILD between patients over and under the age of 48 ($P = 0.667$). The course of disease had no correlation with whether ILD occurred ($P = 0.510$). There was no difference in follow up time between the two groups ($P = 0.183$). Our data showed that patients in the ILD absence group were more likely to have dysphagia and myalgia ($P = 0.000$ and $P = 0.000$). Our results showed that the three symptoms of cough, sputum, and shortness of breath were significantly increased in patients with DM in the ILD presence group, and the difference was statistically significant compared with the ILD absence group ($P = 0.000$). CK and LDH levels did not differ significantly between the groups ($P = 0.328$ and $P = 0.753$, respectively), and there was no relationship between ILD and presence of malignant tumour ($P = 0.193$) in either group. The mortality in ILD group was significantly higher than that in non-ILD group ($P = 0.011$).

Records of the 153 patients with DM were reviewed for positive rates of the twelve types of MSAs and MAAs. There were significant differences in the association of *anti*-Ro52 antibodies ($P = 0.000$), anti-ARS antibodies ($P = 0.000$), and *anti*-MDA5 antibodies ($P = 0.000$) with the incidence of ILD between the two groups, indicating that *anti*-Ro52, anti-ARS and *anti*-MDA5 antibodies may be risk factors associated with ILD development in DM. However, *anti*-TIF1-γ antibodies ($P = 0.000$), *anti*-Mi-2α antibodies ($P = 0.027$), and all antibodies with a negative ($P = 0.001$) may be protective factors for ILD development in patients with DM (Table 1).

3.2. Status of anti-Ro52 antibodies co-expressed with other MSAs and/or MAAs

In 153 cases of patients with DM, 81 (52.9%) were positive for *anti*-Ro52 antibodies. Of them, 14 (9.2%) expressed isolated *anti*-Ro52 antibodies, and the remaining 67 (43.8%) patients expressed a combination of *anti*-Ro52 antibodies and other MSAs and/or MAAs. Of these cases, 20 were positive for a combination of anti-ARS and *anti*-Ro52 antibodies. Four patients were positive for a combination of anti-ARS, *anti*-Ro52, and *anti*-MDA5 antibodies. A single patient was positive for anti-ARS and *anti*-MDA5 antibodies. A total of 23 cases of the 36 positives had a combination of *anti*-MDA5 and *anti*-Ro52 antibodies

Table 1
Patient characteristics of 153 patients with DM stratified by ILD.

Patient Characteristic	ILD		P-value	Total/ Positive(%)
Total (N = 153)	Absence(N = 73)	Presence(N = 80)		
Female/Male	49/24	57/23	0.580	106/47
Age at onset, mean (SD), years	46.19 ± 13.43	50.1 ± 13.45	0.074	48.24 ± 13.54
Age at onset > 48 year (N/Y)	28/45	28/52	0.667	56/97
Course of disease (S.D.), months	23.94 ± 38.46	20.42 ± 27.16	0.510	22.10 ± 32.98
Follow up time, mean (S.D.), years	2.45 ± 2.29	2.00 ± 1.88	0.183	2.21 ± 2.09
Dysphagia (N), n(%)	37(50.7%)	16(20%)	**0.000**	53(34.6%)
Myalgia (N), n(%)	70(95.9%)	56(70%)	**0.000**	126 (82.4%)
Cough, sputum, shortness of breath (N), n(%)	21(28.8%)	61(76.3%)	**0.000**	82(53.6%)
Elevated CK (N), n(%)	34(46.6%)	31(38.8%)	0.328	65(42.5%)
Elevated LDH(N), n(%)	51(63.0%)	54(73.8%)	0.753	105 (68.6%)
Malignant tumour (N), n (%)	9(12.3%)	5(6.25%)	0.193	14(9.2%)
Death(N), n(%)	5(2.7%)	10(16.3%)	**0.011**	15(9.8%)
Anti-Ro52 antibody (N), n (%)	26(35.6%)	55(68.8%)	**0.000**	81(52.9%)
Anti-PM/Scl100 antibody (N), n(%)	3(5.5%)	4(5.0%)	1.000	7(4.6%)
Anti-PM/Scl75 antibody (N), n(%)	4(5.5%)	8(10.0%)	0.461	12(7.8%)
Anti-SRP antibody (N), n (%)	6 (8.2%)	12(15.0%)	0.194	18(11.8%)
Anti-Ku antibody (N), n (%)	5 (6.8%)	4(5.0%)	0.887	9(5.9%)
Total anti-ARS antibodies (N), n(%)	6(8.2%)	32(40%)	**0.000**	38(25.5%)
Anti-TIF1-γ antibody (N), n(%)	26 (35.6%)	8(10.0%)	**0.000**	34(22.9%)
Anti-Mi-2α antibody (N), n (%)	8 (11.0%)	1(1.3%)	**0.027**	9(5.9%)
Anti-Mi-2β antibody (N), n (%)	9 (12.3%)	8(10.0%)	0.647	17(11.1%)
Anti-MDA-5 antibody (N), n(%)	5 (6.8%)	31(38.8%)	**0.000**	36(23.5%)
Anti-SAE-1 antibody (N), n (%)	1(1.4%)	2(2.5%)	1.000	3(2.0%)
Anti-NXP-2 antibody (N), n(%)	11 (15.1%)	8(10.0%)	0.342	19(12.4%)
All antibody negative(N), n(%)	11 (15.1%)	1(1.3%)	**0.001**	12(7.8%)

(Table 2).

3.3. Logistic regression analysis to test the influence factors of ILD

After the initial antibody review, we used twelve types of MSAs and MAAs as logistic regression variables for determining the factors influencing ILD in 153 adult inpatients with DM. *Anti*-Ro52, anti-ARS and *anti*-MDA5 antibodies were found to be risk factors for ILD development (OR = 3.106, $P = 0.013$; OR = 16.588, $P = 0.000$; OR = 10.445, $P = 0.000$; respectively). *Anti*-TIF1-γ antibody was a protective factor for ILD (OR = 0.150, $P = 0.008$), while other MAAs and MSAs were not correlated with ILD development (Table 3).

3.4. HRCT scores of ILD in 153 patients with DM stratified by anti-MDA5, anti-Ro52 antibodies, and/or anti-ARS antibodies

We evaluated the HRCT scores of the 153 inpatients with DM and found that the median score was 50, with the actual scores falling between a minimum of 0 and a maximum of 535. The scores were then divided into 3 categories: normal (≤10), median (>10 and ≤ 50), and

Table 2
Status of *anti*-Ro52 antibodies co-expressed with other MSAs and/or MAAs.

MSAs/MAAs	Total/ Positive(%)	Co-expressed with *anti*-Ro52 antibody
Anti-PM/Scl100 antibody (N), n(%)	7(4.6%)	4(2.6%)
Anti-PM/Scl75 antibody (N), n(%)	12(7.8%)	8(5.3%)
Anti-SRP antibody (N), n(%)	18(11.8%)	10(6.5%)
Anti-Ku antibody (N), n(%)	9(5.9%)	5(3.3%)
Total Anti-ARS antibodies (N), n(%)	38(24.8%)	24(15.7%)
Isolated anti-ARS antibodies (N), n(%)	13(8.5%)	–
Anti-ARS and *anti*-MDA5 antibody (N), n(%)	1(0.7%)	–
Anti-ARS and *anti*-Ro52 and *anti*-MDA5 antibody (N), n(%)	4(2.6%)	–
Anti-ARS and *anti*-Ro52 antibody (N), n(%)	20(13.1%)	–
Anti-Jo-1antibody (N), n(%)	15(9.8%)	7(4.6%)
Anti-PL-7 antibody (N), n(%)	13(8.5%)	7(4.6%)
Anti-PL-12 antibody (N), n(%)	4(2.6%)	1(0.7%)
Anti-EJ antibody (N), n(%)	5(3.3%)	4(2.6%)
Anti-OJ antibody (N), n(%)	2(1.3%)	1(0.7%)
Anti-TIF1-γ antibody (N), n(%)	34(22.2%)	15(9.8%)
Anti-Mi-2α antibody (N), n(%)	9(5.9%)	1(0.7%)
Anti-Mi-2β antibody (N), n(%)	17(11.1%)	9(5.9%)
Anti-MDA-5 antibody (N), n(%)	36(23.5%)	27(17.6%)
Isolated *anti*-MDA5 antibody (N), n (%)	8(5.3%)	–
Anti-Ro52 and *anti*-MDA5 antibodies (N), n(%)	23(15%)	–
Anti-ARS and *anti*-Ro52 and *anti*-MDA5 antibodies (N), n(%)	4(2.6%)	–
Anti-ARS and *anti*-MDA5 antibodies (N), n(%)	1(0.7%)	–
Anti-SAE-1 antibody (N), n(%)	3(2.0%)	0(0%)
Anti-NXP2 antibody (N), n(%)	19(12.4%)	7(4.6%)

Table 3
Logistic regression analysis to test the influence factors of ILD.

Variables	OR	95%CI	P-value
Anti-Ro52 antibody	3.106	1.269–7.604	**0.013**
Anti-PM/Scl100 antibody	1.491	0.216–10.286	0.685
Anti-PM/Scl75 antibody	1.995	0.363–10.539	0.436
Anti-SRP antibody	2.014	0.405–10.022	0.393
Anti-Ku antibody	0.379	0.060–2.396	0.302
Total anti-ARS antibody	16.588	4.912–56.021	**0.000**
Anti-TIF1-γ antibody	0.150	0.037–0.614	**0.008**
Anti-Mi-2α antibody	0.343	0.028–4.242	0.405
Anti-Mi-2β antibody	0.532	0.135–2.107	0.369
Anti-MDA-5 antibody	10.445	3.092–35.285	**0.000**
Anti-SAE-1 antibody	5.545	0.192–160.194	0.318
Anti-NXP-2 antibody	1.432	0.431–4.753	0.558

severe (>50). *Anti*-Ro52, anti-ARS, and *anti*-MDA5 antibodies were found to be risk factors for ILD development (Tables 1 and 3). In the *anti*-Ro52-positive group, only 14 cases were positive with isolated *anti*-Ro52 antibodies, and these patients were negative for other antibodies. The HRCT scores of ILD of the *anti*-Ro52-positive group were significantly higher than those of the *anti*-Ro52-negative group ($P < 0.001$). HRCT scores of ILD in the group with isolated *anti*-Ro52 antibodies were not significantly different from those of the *anti*-Ro52-positive group ($P = 0.674$).

Isolated *anti*-MDA5 antibodies were positive in 8 (5.2%) patients, while in 23 patients, *anti*-MDA5 antibodies were co-expressed with *anti*-Ro52 antibodies. When patients with DM co-expressed both *anti*-MDA5 and *anti*-Ro52 antibodies, the proportion of their HRCT scores in the severe category was 39.1%, which was higher than that of the isolated *anti*-MDA5 antibody group (0%). Therefore, when both *anti*-Ro52 and *anti*-MDA5 antibodies were present, the HRCT scores of ILD increased ($P = 0.018$). Meanwhile, the HRCT scores of the isolated *anti*-MDA5 antibodies group were similar to those of the isolated *anti*-Ro52 group ($P =$

0.172).

Conversely, isolated anti-ARS antibodies were positive in 13 patients, while anti-ARS antibodies were co-expressed with *anti*-Ro52 antibodies in 20 patients. In patients with DM that co-expressed *anti*-Ro52 and anti-ARS antibodies, the HRCT score of ILD was not significantly different from that of the isolated anti-ARS antibodies group (P = 0.888). In the same way, the HRCT scores of the isolated anti-ARS antibodies group was not significantly different from those of the isolated *anti*-Ro52 group (P = 0.361). The HRCT scores of the isolated anti-ARS antibodies group were higher than those of the isolated *anti*-MDA5 group (P = 0.036). Furthermore, the HRCT scores of the isolated anti-ARS antibodies group were not different from those of the *anti*-MDA5 antibodies co-expressed with *anti*-Ro52 antibodies group (P = 0.633) (Table 4).

3.5. : Kaplan-Meier survival curve for the survival and death cases

Based on the positive-antibody of death cases, we choose the *anti*-Ro-52, *anti*-MDA5, anti-ARS and *anti*-TIF1-γ antibodies for the Kaplan-Meier survival curve analysis. The results showed that patients with DM positive with *anti*-Ro52 and *anti*-MDA5 antibodies had worse prognosis (Fig. 1A and B, P = 0.005 and P = 0.001). While, there was no correlation between the expression of anti-ARS and *anti*-TIF1-γ antibodies and the survival time of patients with DM (Fig. 1C and D, P = 0.932 and P = 0.388). In addition, the patients expressed with *anti*-MDA5 antibodies develops rapidly, showing the characteristics of short course of disease and high mortality. Interesting, patients with DM were positive with *anti*-Ro52 and *anti*-MDA5 antibodies has worse outcome than the patients positive with *anti*-Ro52 or *anti*-MDA5 antibodies.

Table 4
HRCT scores of ILD in 153 patients with DM stratified by *anti*-MDA5, *anti*-Ro52 antibodies, and/or anti-ARS antibodies.

Antibody	Total	ILD Score of CT			P-value
	N = 153	Normal (Score≤10)	Median (Score ≤50)	Severe (Score>50)	
anti-Ro52+	N = 81	26 (32.1%)	29 (35.8%)	26 (32.1%)	**0.000**
anti-Ro52-	N = 72	47(65.3%)	17(23.6%)	8 (11.1%)	
anti-Ro52+	N = 81	26 (32.1%)	29 (35.8%)	26 (32.1%)	0.674
Isolated *anti*-Ro52+	N = 14	6(42.3%)	5(35.7%)	3(21.4%)	
Isolated *anti*-MDA5+	N = 8	3(87.5%)	5 (62.5%)	0(0%)	**0.018**
anti-MDA5+ and *anti*-Ro52+	N = 23	2(8.7%)	12 (52.2%)	9(39.1%)	
Isolated anti-ARS+	N = 13	3(23.1%)	5(38.4%)	6(46.2%)	0.888
Anti-ARS + *anti*-Ro52+	N = 20	3(15%)	8(40%)	9(45%)	
Isolated *anti*-Ro52+	N = 14	6(42.3%)	5(35.7%)	3(21.4%)	0.172
Isolated *anti*-MDA5+	N = 8	3(87.5%)	5 (62.5%)	0(0%)	
Isolated *anti*-Ro52+	N = 14	6(42.3%)	5(35.7%)	3(21.4%)	0.361
Isolated anti-ARS+	N = 13	3(23.1%)	5(38.4%)	6(46.2%)	
Isolated anti-ARS+	N = 13	3(23.1%)	5(38.4%)	6(46.2%)	**0.036**
Isolated *anti*-MDA5+	N = 8	3(87.5%)	5 (62.5%)	0(0%)	
anti-MDA5+ and *anti*-Ro52+	N = 23	2(8.7%)	12 (52.2%)	9(39.1%)	0.633
Isolated anti-ARS+	N = 13	3(23.1%)	5(38.4%)	6(46.2%)	

(Fig. 1E, P = 0.001). The specific clinical features of 15 cases of death patients were detailed in Supple.2.

4. Discussion

MSAs and MAAs have a certain predictive value for the prognosis of DM [10]. In China, the largest hospitals began to fully screen for MSAs and MAAs in DM patients in 2016, and this study retrospectively analysed the records of 153 inpatients with DM. We analysed the positive rate of MSAs and MAAs, collected data on the incidence of ILD, screened for cancer, calculated the HRCT score, and analysed the relationship between the MSAs, MAAs, and ILD.

Previous studies have reported that *anti*-Jo-1 and anti-PL-7 antibodies show the highest positive rates among the anti-ARS antibodies in DM, with the positive rates in patients with DM being less than 10% [11–13]. Our data showed that the positive rates of *anti*-Jo-1 and anti-PL-7 antibodies were 9.8% and 8.5%, respectively. However, the positive rates of the other anti-ARS antibodies were lower, such as anti-PL-12, *anti*-EJ, and *anti*-OJ antibodies, which were 2.6%, 3.3%, and 1.3%, respectively. These results were similar to previous reports [14]. ILD is the most common and dominant organ involvement in Jo-1 antibody associated anti-ARS antibodies, and is associated with increased incidence rate and mortality [15]. However, in our study, 15 of the 153 patients with DM were *anti*-Jo-1 antibodies positive. There was no difference between the *anti*-Jo-1 antibodies positive group and the *anti*-Jo-1 antibodies negative group in the development of ILD. This phenomenon may be caused by too few patients being positive for *anti*-Jo-1 antibodies. In the future, we will increase the sample size to reassess the effect of *anti*-Jo-1 antibodies on ILD. When we combined the 5 types of anti-ARS antibodies into a total anti-ARS antibodies group for analysis, the predictive value of anti-ARS antibodies on ILD development is noteworthy in DM. Our study showed that the *anti*-TIF1-γ antibody may be a protective factor for ILD in DM. These results were similar to those of a previous study [16]. Further studies are required, with increased sample sizes and extended follow-up time, to confirm this phenomenon.

Anti-MDA5 antibodies are often associated with rapidly progressive ILD, as confirmed by a previous study [17]. Our study also found that patients expressed with *anti*-MDA5 antibodies had a short course of disease and high mortality. Meanwhile, of the 153 patients with DM, 23 co-expressed *anti*-MDA5 and *anti*-Ro52 antibodies and only 8 presented with isolated *anti*-MDA5 antibodies. This result indicates that most patients with DM co-express *anti*-MDA5 and *anti*-Ro52 antibodies. Our study also showed that the HRCT score of ILD increased when both *anti*-Ro52 and *anti*-MDA5 antibodies are present. This phenomenon may suggest that when the *anti*-MDA5 and *anti*-Ro52 antibodies are both present, we should be highly alert to the emergence of severe ILD.

Our data showed that *anti*-Ro52 antibody was the most common antibodies, with a positive rate of 52.9%. Logistic regression analysis indicated that *anti*-Ro52 antibody was a risk factor for ILD in patients with DM. Meanwhile, HRCT scores of the isolated anti-ARS antibodies group or isolated *anti*-MDA5 antibodies group were not higher than those of the isolated *anti*-Ro52 group. Kaplan-Meier survival curve analysis showed that patients with DM positive with *anti*-Ro52 antibody had poor prognosis. Previous studies have also shown that anti–SSA/Ro52 or any anti-ARS antibodies or both might be affect the prognosis of DM/PM [18].

Anti-Ro52 antibodies were previously neglected antibody in patients with DM. Previous studies showed that *anti*-Ro52 antibodies were involved in transcription regulation, displays pathogenicity, but lacks specificity, and could appear in many connective tissue diseases [19]. *Anti*-Ro52 antibodies could cause lupus and atrioventricular blockage in neonates through the placental barrier [20]. One study found that the risk of developing ILD is higher in the presence of *anti*-Ro52 antibodies in primary Sjogren's syndrome [21]. A nationwide cross-sectional study in Norway suggested that *anti*-Ro52 antibodies were significantly

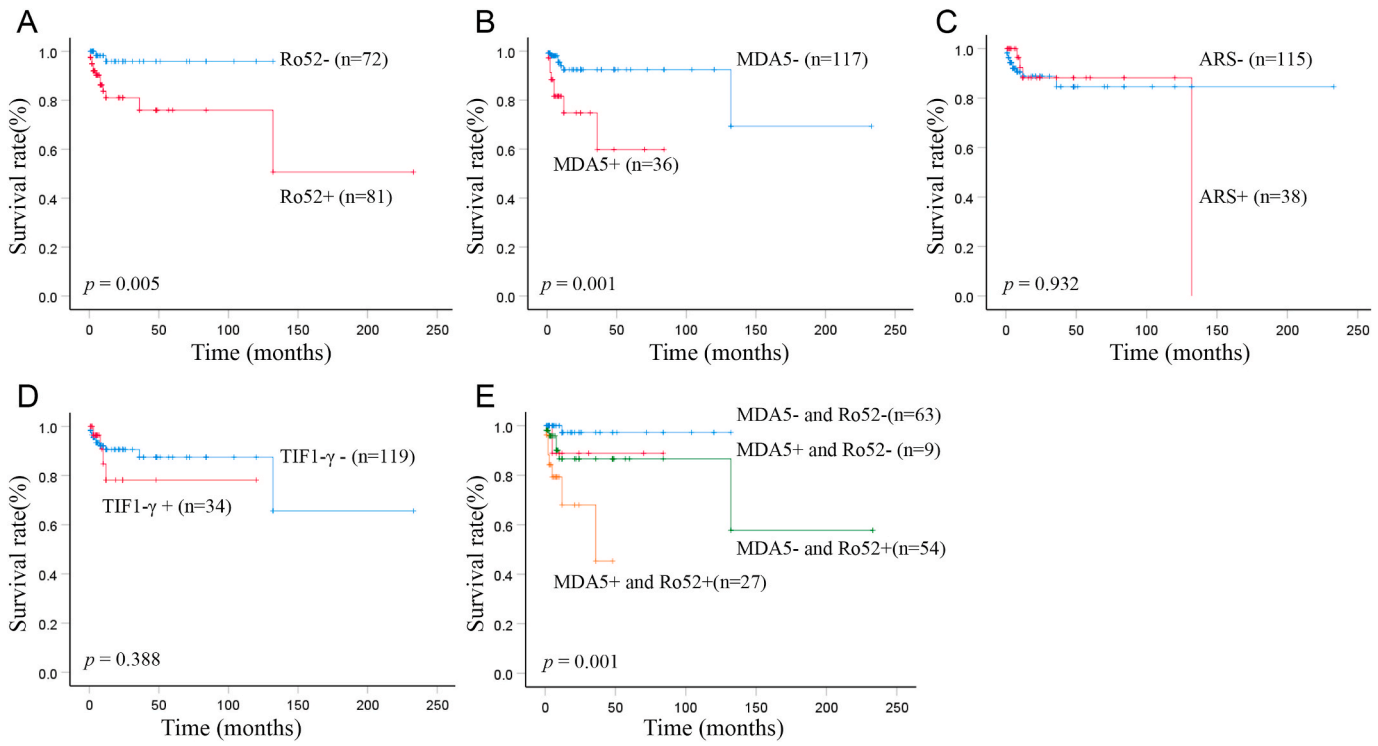

Fig. 1. Kaplan-Meier survival curve for the survival and death cases

associated with the occurrence of pulmonary fibrosis in mixed connective tissue disease; thus, *anti*-Ro52 antibodies could also be used as an indicator for predicting the occurrence of ILD [22]. A report showed that the *anti*-Ro52 antibodies were related to pulmonary fibrosis DM in children, and to the severity of the disease [6]. However, the severity of DM is difficult to evaluate as systemic and opportunistic infections in patients with DM have become major influencing factors in DM prognosis, rather than only being associated with malignant tumour or ILD [1]. Therefore, we speculated that *anti*-Ro52 antibodies are a risk factor for ILD, regardless of the type of connective tissue disease. However, this needs to be verified by further clinical studies of connective tissue disease.

5. Conclusion

In conclusion, our data showed that *anti*-Ro52 antibodies were the most commonly found antibodies in 153 patients with DM, with a positive rate of 52.9%. We also found that *anti*-Ro52 antibodies had a predictive effect on ILD development and are an independent risk factor for ILD in patients with DM. Patients with DM positive with *anti*-Ro52 and *anti*-MDA5 antibodies had worse prognosis. These result requires further verification with data obtained by studying larger cohorts with long-term patient follow-ups.

CRediT authorship contribution statement

Xiaojing Xing: Methodology, Data curation, Formal analysis, Writing - original draft. **Anqi Li:** Data curation, /evidence collection, Validation. **Chengxin Li:** Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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